American Journal of Hematology 56:248–251 (1997)

Parvovirus B19 Quiescence During the Course of Human Immunodeficiency Virus Infection in Persons With Hemophilia

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To detect and characterize parvovirus B19 infection during the course of progressive immune deficiency from human immunodeficiency virus (HIV), ten subjects enrolled in the Multicenter Hemophilia Cohort Study were followed for 6.4 to 15 years from HIV seroconversion through extreme immune deficiency. Four to five sera or plasma samples from each subject, collected at predetermined CD4⁺ lymphocyte levels, were tested for immunoglobulin G (IgG) and M (IgM) B19 antibodies and DNA. All 42 samples were positive for B19 IgG antibodies, and three were weakly positive for IgM antibodies. Only one sample, collected coincident with HIV seroconversion, was unequivocally positive for B19 DNA. No persistent hematologic adverse effects of B19 infection were observed. Thus, although B19 IgG antibodies are highly prevalent among HIV-infected persons with hemophilia or related disorders, B19 viremia and its hematologic consequences were not detected, even with severe depletion of CD4⁺ lymphocytes. If primary B19 infection occurs after immune deficiency, however, the consequences may be more adverse. Am. J. Hematol. 56:248–251, 1997.

Key words: parvovirus B19; human immunodeficiency virus; AIDS; hemophilia; polymerase chain reaction; prospective cohort study

INTRODUCTION

Among patients with the acquired immunodeficiency syndrome (AIDS), infection with human parvovirus B19 has been noted as a cause of severe anemia that can be reversed by treatment with intravenous immunoglobulin [1]. The possibility that B19 could be yet another AIDS-associated opportunistic infection has caused concern, especially among persons with hemophilia, nearly all of whom became infected with B19 through use of clotting factor concentrates [2–6]. However, reactivation or persistence of B19 in this population remains unclear. Therefore, we prospectively evaluated a series of ten persons with clotting disorders during the course of progressive HIV-1-related immune deficiency for B19 viremia, anemia, or other related diseases.

METHODS

Among 1215 HIV-1-infected subjects in the Multicenter Hemophilia Cohort Study, 408 have developed an

The authors are members of the Multicenter Hemophilia Cohort Study.

Contract grant sponsor: National Cancer Institute; Contract grant numbers: NO1-CP-33002, NO1-CP-33060.

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Received for publication 16 January 1997; Accepted 9 July 1997

AIDS-defining clinical condition or a CD4$^+$ lymphocyte level below 50 cells/μL [7,8]. To estimate B19 prevalence and incidence at specific levels of immunity, the current study focused on 37 of these 408 subjects who had frozen serum or plasma from at least two blood draws at each of four CD4$^+$ lymphocyte levels defined a priori (500 or more cells/μL, 200 to 499 cells/μL, 50 to 199 cells/μL, and after AIDS or fewer than 50 cells/μL). To minimize possible differences between centers, the three largest centers were selected, representing 18 subjects. Of these 18 subjects, we selected those 10 who had the largest stored serum or plasma volumes at all four CD4 levels and who represented a cross-section of ages.

Sera or plasma samples were tested for B19 IgG and IgM antibodies by capture immunoassay [9]. B19 DNA was detected by polymerase chain reaction (PCR) assay previously described, which utilizes primers P1 and P6 to a highly conserved region of the 77 kDa non-structural protein gene, NS1 [10]. PCR products were sized by gel electrophoresis and ethidium bromide staining. All negative primary reactions were reamplified using a nested set of primers, P2 and P5; the combined procedures typically detect fewer than 10 B19 genome copies in a specimen sample. To confirm that positive reactions were due to the presence of B19 genome, and not contaminating PCR product from the preceding reactions, all positive specimens were confirmed with a second set of primers to the 84 kDa structural protein gene, VP1. Because heparin is a recognized inhibitor of Taq polymerase, plasma samples were tested both before and after treatment with heparinase I (Sigma Chemical Co., St. Louis, MO) [11]. Samples also were spiked with a B19 vector, which was diluted to a final concentration of less than 100 genome copies and amplified as a positive control.

RESULTS

Forty-two samples from ten hemophilic subjects were evaluated for parvovirus B19 antibodies and DNA; at least one sample from each subject was tested at each CD4$^+$ lymphocyte stratum (Table I). The earliest samples were collected from subjects aged 5 to 44, immediately before to 5.7 years after HIV-1 seroconversion. Follow-up ranged from 6.4 to 15 years after HIV-1 seroconversion. Seven subjects had moderate or severe hemophilia A (three with factor VIII inhibitors), two had severe hemophilia B, and one had severe von Willebrand's disease.

During the study period (May 1984 to January 1995), the ten subjects received a wide range of clotting factor products, before and subsequent to implementation of viral inactivation procedures. Zidovudine therapy was initiated in all ten subjects between December 1988 and February 1991. Other antiretroviral drugs were given later. Acyclovir was used by three subjects, starting in March 1991 to November 1993. No other antiviral drugs were reported used during the study period. Intravenous immunoglobulin was given only to subject 5, in September and October 1991.

All 42 samples were positive for B19 IgG antibodies. Three samples were weakly positive for IgM antibodies, including the second and third from subject 4 and the third from subject 5, when their CD4 counts ranged from 86 to 286 cells/μL (Table I). During this time they were receiving zidovudine and factor VIII concentrate purified by immunoaffinity absorption. B19 DNA was not detected in these two subjects. Subject 4's hematocrit declined from 46 to 39%, but similar hematocrit declines were observed in other subjects who did not have IgM antibodies.

By PCR, none of the 42 samples was positive for B19 DNA. Using the more sensitive nested PCR technique, one sample (the first from subject 1) was repeatedly positive with the NS1 and the VP1 primers (Table I). Two other samples were initially reactive with the NS1 primers but were negative when repeated with the NS1 primers and also were negative with the VP1 primers. The remaining 39 samples were repeatedly negative. The three PCR-reactive samples were drawn when the subjects were 11 to 12 years of age and before the onset of severe immune deficiency. During this time, they were receiving clotting factor concentrates, and subject 3 was receiving zidovudine.

Subject 1, a 12-year-old boy who required chronic iron replacement for intermittent gastrointestinal bleeding, was repeatedly positive for B19 DNA in May 1984. Two months earlier, in March, he was hospitalized for headache, cough, and vomiting; on examination, he was afebrile but had an exudative tonsillitis and stiff neck. A subdural hematoma was demonstrated by computed tomography, but he recovered quickly with high-dose factor concentrates. In May, he returned with fever (39.6°C), Group A β-hemolytic streptococcal pharyngitis, and upper gastrointestinal bleeding. He also was noted to be pancytopenic (hematocrit 30%; 0.7×10^6 neutrophils/L; 1.0×10^6 lymphocytes/L; 91×10^9 platelets/L). Over the course of 4 days, the fever, pharyngitis, bleeding, leukopenia, and thrombocytopenia resolved with antibiotics and factor concentrates, although he was still anemic (hematocrit 25%) at discharge on day 7. With iron replacement, his hematocrit increased to 38% within 3 months, but at that time his serum had become positive for HIV-1 antibodies.

DISCUSSION

The hemophilic subjects in this study, like most persons treated with plasma-derived clotting factor concentrates, were exposed to human parvovirus B19 on numerous occasions over many years [6]. During the early

TABLE I. Parvovirus B19 Detection During the Course in Human Immunodeficiency Virus Infection Among Ten Patients With Hemophilia*

						Parvovirus		
						B19 antibody		
Patient no.	Date	Age	HIV seroconversion duration (years)	CD4 count (cells/μL)	Hematocrit (%)	IgG	IgM	B19 genome nested PCR
1	5/84	12	−0.1[a]	nd	30[b]	+	−	++
	4/88	16	3.8	302	41	+	−	−
	12/88	16	4.5	75	41	+	−	−
	11/90	18	6.4	16[c]	38	+	−	−
2	11/85	11	1.9	672	36	+	−	(+)[d]
	5/89	14	5.3	276	35	+	−	−
	4/93	18	9.3	116[c]	41	+	−	−
	1/95	20	11.0	43[c]	39	+	−	−
3	8/86	9	2.7	992	37	+	−	−
	5/89	12	5.4	277	37	+	−	(+)[d]
	9/90	13	6.7	50	37	+	−	−
	8/93	16	9.7	12[c]	25	+	−	−
4	11/85	44	4.3	746	46	+	−	−
	9/89	48	8.2	286	46	+	+	−
	10/91	50	10.2	156	39	+	+	−
	5/94	53	12.9	30	32	+	−	−
5	10/88	5	4.0	791	35	+	−	−
	4/90	6	5.5	289	33	+	−	−
	6/91	7	6.7	86	35	+	+	−
	11/93	10	9.1	0	34	+	−	−
6	9/86	8	4.0	519	36	+	−	−
	5/90	12	7.7	411	37	+	−	−
	11/92	14	10.2	130	40	+	−	−
	8/94	16	11.9	25[c]	39	+	−	−
7	11/84	23	5.7	512	43	+	−	−
	7/88	27	9.4	207	42	+	−	−
	9/92	31	13.6	102	37	+	−	−
	3/94	33	15.0	55[c]	27	+	−	−
8	11/85	27	5.0	nd	47	+	−	−
	10/86	28	5.9	750	45	+	−	−
	7/89	31	8.7	389	42	+	−	−
	9/91	33	10.8	60	39	+	−	−
	10/92	34	12.0	120[c]	34	+	−	−
9	9/86	39	2.7	642	44	+	−	−
	10/89	42	5.9	416	43	+	−	−
	5/92	45	8.5	75	44	+	−	−
	7/94	46	10.6	7	44	+	−	−
10	12/85	41	4.1	nd	29	+	−	−
	10/86	42	5.0	1,272	33	+	−	−
	4/88	43	6.5	190	34	+	−	−
	11/89	45	8.1	90[c]	33	+	−	−
	2/91	46	9.3	55[c]	35	+	−	−

*nd = not done.
[a]Before HIV-1 antibody seroconversion. First and second CD4 counts after seroconversion were 546 and 758 cells/μL, respectively.
[b]Concurrent leukopenia and thrombocytopenia, which resolved over 4 days (see Results.)
[c]After an AIDS-defining opportunistic infection or malignancy.
[d]Positive only on initial assay with primers to the NS1 gene; negative on repeat with these primers and with primers to the VP1 gene.

years of the study, they also may have received small amounts of anti-B19 antibodies passively infused with intermediate purity plasma concentrates. The higher purity factor concentrates used during the past several years, however, contain no detectable immunoglobulins.

Chronic B19 infections have been reported in patients receiving cancer chemotherapy or with congenital or acquired immune deficiencies [5,6]. Despite long-term follow-up and very severe cellular immune deficiency, we found no evidence of B19 reactivation or persistent viremia in our ten subjects. As we studied only 10 subjects, we certainly could have missed others who did have B19 viremia. However, the recent study of Ragni et al. [12] found an 82% anti-B19 antibody prevalence but no de-

tectable B19 DNA in a cross-sectional evaluation of 136 hemophilic patients, including 23 anti-B19-positive patients with AIDS, further suggesting that the prevalence of B19 viremia with AIDS is likely to be low.

Humoral immunity may be key to controlling B19 infection, as illustrated by the rapid clearance of viremia and resolution of anemia with intravenous immunoglobulin [1,13]. Because persons with HIV-1 infection often have defective humoral immunity, especially after the onset of severe cell-mediated immune deficiency [14] it is surprising that B19 viremia was seldom detected in our study. With HIV-1 infection, however, humoral responses to recall antigens tend to be relatively well preserved, whereas responses to new antigens often are severely impaired [14]. B19 infection has usually antedated HIV-1 infection among persons with hemophilia. Our data suggest that even with severe HIV-1-related immune deficiency, pre-existent antibodies to B19 protect against reactivation of and subsequent exposures to this virus. Conversely, a primary B19 infection that occurs after HIV-1 may not be well controlled and may result in red-cell aplasia or other serious consequences.

Patient 1 had B19 DNA detected during an illness that may have been an HIV-1 seroconversion syndrome. Perhaps B19 reactivated during primary HIV-1 infection or contributed to his symptomatic illness. Alternatively, the factor concentrate infusions used to treat his subdural hematoma may have transmitted both viruses coincidentally. In either case, the infection had no persistent hematologic effect.

Superficially, our results differ from those of Musiani and colleagues, who detected clinical disorders and B19 DNA in serial samples from four of seven HIV-1-infected hemophilic patients in Italy [15]. The same investigators also reported detecting B19 DNA in 6 to 14% of 49 patients with HIV-1 infection [16]. However, these patients were highly selected, as more than half had IgM antibodies against B19 [16]. In addition, ages and B19 antibody status were not presented for the hemophilia study patients [15]. Thus, they may have detected primary B19 infections among persons with severe HIV-1-related immune deficiency.

In summary, among ten persons with hemophilia with HIV-1 infection who were followed for a median of 10.8 years and to a median of 28 $CD4^+$ lymphocytes/μL, none developed reactivated or persistent B19 viremia. This suggests that B19 IgG antibodies may be sufficient to prevent or control viremia and consequent anemia even in this immunocompromised population. Persons who have severe immune deficiency when they are first infected with B19, however, may have a different outcome.

ACKNOWLEDGMENTS

The authors are grateful to the physicians, nurses, staff, and especially the subjects of the Multicenter Hemophilia Cohort Study for their many years of collaboration. This work was supported in part by National Cancer Institute contracts NO1-CP-33002 with Research Triangle Institute and NO1-CP-33060 with Biotech Research Laboratories, Inc.

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